Ag-Ab reactions
Tests for Ag-Ab reactions

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Importance of Ag-Ab Reactions

- Understand the mechanisms of defense
- Abs as tools in:
  - Treatment
  - Diagnosis
    - As biomarkers
    - As tools to measure analytes
Nature of Ag/Ab Reactions

• Lock and Key Concept

• Non-covalent Bonds
  – Hydrogen bonds
  – Electrostatic bonds
  – Van der Waal forces
  – Hydrophobic bonds

• Multiple Bonds

• Reversible

Affinity

• Strength of the reaction between a single antigenic determinant and a single Ab combining site

Affinity = \(\Rightarrow\) attractive and repulsive forces
Calculation of Affinity

\[ \text{Ag} + \text{Ab} \leftrightarrow \text{Ag-Ab} \]

Applying the Law of Mass Action:

\[ K_{eq} = \frac{[\text{Ag-Ab}]}{[\text{Ag}] \times [\text{Ab}]} \]
Avidity

• The overall strength of binding between an Ag with many determinants and multivalent Abs

\[ K_{eq} = \begin{array}{ccc}
10^4 & 10^6 & 10^{10} \\
\text{Affinity} & \text{Avidity} & \text{Avidity}
\end{array} \]
Specificity

• The ability of an individual antibody combining site to react with only one antigenic determinant.
• The ability of a population of antibody molecules to react with only one antigen.
Cross Reactivity

• The ability of an individual Ab combining site to react with more than one antigenic determinant.

• The ability of a population of Ab molecules to react with more than one Ag
Factors Affecting Measurement of Ag/Ab Reactions

- Affinity
- Avidity
- Ag:Ab ratio
- Physical form of Ag
Do you need to know what happens in Lab.

- Know different sources of random and systematic errors
- Know limitations of current techniques
- Know which instruments are being used
- Know how reliable the results are in clinical decision making
Errors

Requesting appropriate tests
Writing prescription
Reading prescription
Sample collection
Sampling times
environmental factors
Drug interferences
Patient identification
Sample transfer
Technician errors
Instrumental errors
Method limitations
Data entry mistakes
Interpretation of results

How can we trust
What you want

- Accuracy
- Precision
- Speed
- Sensitivity
- Specificity
Spectrophotometry: Luminescence

- Can you tell the difference between how many marks are in each box?

Sensitivity of Absorbance Measurements
Spectrophotometry: Luminescence

- Can you tell the difference between how many marks are in each box?

0

40

Sensitivity of Luminescence Measurements
Lab Tests Based on Ag/Ab Reactions

• All tests based on Ag/Ab reactions will have to depend on lattice formation or they will have to utilize ways to detect small immune complexes
• All tests based on Ag/Ab reactions can be used to detect either Ag or Ab
Antigen antibody tests

- Qualitative
- Semi-quantitative
- Quantitative

- In-vivo
- In-vitro

- End point
- Kinetic
Qualitative/quantitative

- Qualitative
  - determines antigen or antibody is present or absent

- Quantitative
  - determines the quantity of the antibody
  - Titer
  - The highest dilution of the specimen usually serum which gives a positive reaction in the test
Common methods in creating dilutions

0.5 ml transferred from tube to tube

0.5 ml saline per tube

1 ml of antiserum (1:10)

Discard

0.5 ml

Control

1:20 1:40 1:80 1:160 1:320 1:640 1:1280 1:2560

Dr. T.V. Rao MD
Tube testing
Classification of Ag-Ab interactions

1. Primary serological tests: (Marker techniques) e.g.
   - Enzyme linked immuno sorbent assay (ELISA)
   - Immuno florescent antibody technique (IFAT)
   - Radio immuno assay (RIA)

2. Secondary serological tests: e.g.
   - Agglutination tests
   - Precipitation tests
   - Flocculation tests
   - Complement fixation tests (CFT)
   - Serum neutralization tests (SNT)
   - Toxin-antitoxin test

3. Tertiary serological test: e.g.
   - Determination of the protective value of an anti serum in an animal.
Agglutination/hemagglutination

Lattice Formation

- Rapid agglutination test
  - eg, Blood group, CRP, RF
- Standard tube agglutination test
  - eg, wright test, Widal test

- Active
- Passive
- Inhibition
Rapid Agglutination tests
Active Agglutination/Hemagglutination

• Definition - tests that have as their endpoint the agglutination of a particulate antigen
  – Agglutinin/hemagglutinin

• Qualitative agglutination test
  – Ag or Ab
### Agglutination/Hemagglutination

- **Quantitative agglutination test**
  - Titer
  - Prozone

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**Titer**
- 64
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- 512
- <2
- 32
- 128
- 32
- 4
Agglutination/Hemagglutination

• Applications
  – Blood typing
  – Bacterial infections
    – Fourfold rise in titer

• Practical considerations
  – Easy
  – Semi-quantitative
Passive Agglutination/Hemagglutination

- **Definition** - agglutination test done with a soluble antigen coated onto a particle

- **Applications**
  - Measurement of antibodies to soluble antigens
Coombs (Antiglobulin) Tests

• Direct Coombs Test
  – Detects antibodies on erythrocytes
Coombs (Antiglobulin) Tests

• Indirect Coombs Test
  – Detects anti-erythrocyte antibodies in serum

**Step 1**

- Patient’s Serum
- Target RBCs

**Step 2**

- Coombs Reagent (Antiglobulin)
Applications of Coombs (Antiglobulin) Tests

- Detection of anti-Rh Ab
- Detection of Incomplete Ab
- Autoimmune hemolytic anemia
Agglutination/Hemagglutination Inhibition

- **Definition** - test based on the inhibition of agglutination due to competition with a soluble Ag

**Prior to Test**

\[ \text{Y} + \text{Ag} \leftrightarrow \text{Y} \]

**Test**

\[ \text{Y} + \text{Ag} + \text{HCG} \leftrightarrow \text{Y} \]

Patient’s sample
Precipitation tests

Lattice Formation

• The antigen and antibody are in soluble form
• Combine to form a visible precipitate
• Presence of electrolytes
• Positive controls and negative controls
LAB tests based on Precipitation

• In gel or solid support
  • eg, Immunodiffusion (Single radial, Double), Immunochromatography, immunofixation electrophoresis, Immunoelectrophoresis, Countercurrent electrophoresis (CCEP),

• In solution
  • eg, Ring test, Turbidimetry, Nephelometry
Radial Immunodiffusion (Mancini)

• **Method**
  – Ab in gel
  – Ag in a well

• **Interpretation**
  – Diameter of ring is proportional to the concentration

• **Quantitative**
  – Ig levels
Immunoelectrophoresis

• Method
  – Ags are separated by electrophoresis
  – Ab is placed in trough cut in the agar

• Interpretation
  – Precipitin arc represent individual antigens
Countercurrent electrophoresis

• Method
  – Ag and Ab migrate toward each other by electrophoresis
  – Used only when Ag and Ab have opposite charges

• Qualitative
  – Rapid
Immunochromatography

Absorbing pad

Reaction pad

Control line

Test line

Conjugated pad

Sample pad

Control line

Test line

Sample
Immuno-precipitation in solution

- Ring test
- Turbidimetry
- PET
- Nephelometry
Light reflection
Molecular size and scattering
Nephelometry vs. Turbidimetry

0°-90°
Flocculation

- Ags are lipids
  - eg, VDRL, RPR

Slide test - Flocculation test – VDRL test

- VDRL Test – a drop of VDRL antigen to a drop of patients serum,
- Shake
- The reaction observed under microscope
- Observe for flocculation reaction
- A Khan test is done in a test tube
Test based on Primary Ag-Ab reaction

- Lattice formation not required
- How can be visualized??

Detection of cell associated analytes:
  - Immunofluorescence
  - Flowcytometry

Detection of soluble Analytes:
  - Radioimmunoassays (RIA)
  - Enzyme-Linked Immunosorbent Assays (ELISA)
Immunoassays basics

1. Bind first antibody to well of microtiter plate
2. Add varying amount of antigen
3. Remove unbound antigen by washing
4. Add labeled second antibody specific for nonoverlapping epitopes of antigen
5. Remove unbound labeled second antibody by washing; measure amount of second antibody bound
Indirect versus direct (sandwich) ELISA

(a) Indirect ELISA

1. Antigen-coated well
2. Add specific antibody to be measured
3. Add enzyme-conjugated secondary antibody
4. Add substrate (S) and measure color

(b) Sandwich ELISA

1. Antibody-coated well
2. Add antigen to be measured
3. Add enzyme-conjugated secondary antibody
4. Add substrate and measure color
Why ELISA can't be fully automated
Competitive RIA/ELISA for Ag

- Method cont.
  - Determine amount of labeled Ag bound to Ab

  ![Diagram of Competitive RIA/ELISA]

  - Concentration determined from a standard curve using known amounts of unlabeled Ag

- Quantitative
  - Most sensitive test
Tests for Cell Associated Antigens

Lattice formation not required
Phosphorescence
Chemiluminescence
Bioluminescence
Immunofluorescence

- Direct
  - Ab to tissue Ag is labeled with fluorochrome
Immunofluorescence

- **Indirect**
  - Ab to tissue Ag is unlabeled
  - Fluorochrome-labeled anti-Ig is used to detect binding of the first Ab.

- **Qualitative to Semi-Quantitative**
Fluorescence

FITC + UV light
Immunofluorescence

• Flow Cytometry
  – Cells in suspension are labeled with fluorescent tag
    • Direct or Indirect Fluorescence
      – Cells analyzed on a flow cytometer
Immunofluorescence

- Flow Cytometry cont.
  - Data displayed

One Parameter Histogram

- Unstained cells
- FITC-labeled cells

Green Fluorescence Intensity

Two Parameter Histogram

- Green Fluorescence Intensity
- Red Fluorescence Intensity
Assays Based on Complement

Lattice formation not required
Complement Fixation

- **Methodology**
  - Ag mixed with test serum to be assayed for Ab
  - Standard amount of complement is added
  - Erythrocytes coated with Abs is added
  - Amount of erythrocyte lysis is determined

![Diagram](image)
Relative sensitivities of tests (approx)

Usual operating range
[Ab] or [Ag]

precipitation
immunoelectrophoresis

double/radial diffusion \[10 \, \mu g/ml \text{ - } 1 \, \text{mg/ml}\]

immunofluorescence \[0.1 \text{ - } 10 \, \mu g/ml\]

ELISA (colour)

(chemiluminescence) \[0.01 \text{ - } 10 \, \text{ng/ml}\]

radioimmunoassay \[0.01 \text{ - } 10 \, \text{ng/ml}\]